1993a, 1993b; Dubey et al., 1994; Dubey, Goodwin et al., 1995), and it is reasonable to believe that the results of this study are accurate. Orosz et al. (1992) reported MAT titers of 1:4,096 in an adult male cassowary (*Casuarius casuarius*) and in an 8-mo-old rhea (*Rhea americana*) suspected to have clinical toxoplasmosis. Two other cassowaries and 3 ostriches on the same farm tested negative for MAT *T. gondii* antibodies. The rheas look similar to ostriches but are smaller than ostriches.

To our knowledge, this is the first record of *T. gondii* infection in ostriches. Although the parasite has been found in various species of birds and in eggs, toxoplasmosis is not recognized as an animal health problem in any species of farmed birds. Similarly, the consumption of avian meat or eggs has not been considered as a likely source of infection for *T. gondii* in people (Dubey and Beattie, 1988). The results of this study demonstrate a low prevalence of *T. gondii* infection in farmed ostriches and suggest that consumers are unlikely to acquire toxoplasmosis from ostrich meat. Testing of meat for the actual presence of *T. gondii* can be accomplished by the use of bioassays or DNA probes (Dubey and Beattie, 1988; MacPherson and Gajadhar, 1993).

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Experimental Transmission of *Sarcocystis speeri* Dubey and Lindsay, 1999 from the South American Opossum (*Didelphis albiventris*) to the North American Opossum (*Didelphis virginiana*)

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ABSTRACT: *Sarcocystis speeri* Dubey and Lindsay, 1999 from the South American opossum *Didelphis albiventris* was successfully transmitted to the North American opossum *Didelphis virginiana.* Sporocysts from a naturally infected *D. albiventris* from Argentina were fed to 2 γ-interferon knockout (KO) mice. The mice were killed 64 and 71 days after sporocyst feeding (DAF). Muscles containing sarcocysts from the KO mouse killed 71 DAF were fed to a captive *D. virginiana*; this opossum shed sporocysts 11 days after ingesting sarcocysts. Sporocysts from *D. virginiana* were fed to 9 KO mice and 4 budgerigars (*Melopsittacus undulatus*). Schizonts, sarcocysts, or both of *S. speeri* were found in tissues of all 7 KO mice killed 29–85 DAF; 2 mice died 39 and 48 DAF were not necropsied. *Sarcocystis* stages were not found in tissues of the 4 budgerigars fed *S. speeri* sporocysts and killed 35 DAF. These results indicate that *S. speeri* is distinct from *Sarcocystis falcatula* and *Sarcocystis neurona,* and that *S. speeri* is present in both *D. albiventris* and *D. virginiana.*

The North American opossum is a host for at least 3 pathogenic species of *Sarcocystis*: *Sarcocystis falcatula* (Box and Duszynski, 1978; Duszynski and Box, 1978; Box et al., 1984; Marsh et al., 1997), *Sarcocystis neurona* (Dubey et al., 1991; Fenger et al., 1997; Dubey and Lindsay, 1998), and *Sarcocystis speeri* (Dubey et al., 1998; Dubey and Lindsay, 1999). Recently, *S. speeri*-like organisms were found in the South American opossum, *Didelphis albiventris* from Argentina (Dubey, Venturini et al., 2000). In the present paper, we present evidence that an *S. speeri*-like organism based on morphology from *D. albiventris* is transmissible and infective to *Didelphis virginiana.*

Gamma-interferon knockout (KO) mice (BALB/c-Ifng^{tm1Ts}) were obtained from Jackson Laboratories (Bar Harbor, Maine). The budgerigars (*Melopsittacus undulatus*) used were obtained from a local aviary. Two experiments were performed.

In experiment 1, sporocysts from opossum 1 (*D. albiventris*) from Argentina were fed to 2 KO mice (nos. 4217, 4218) and

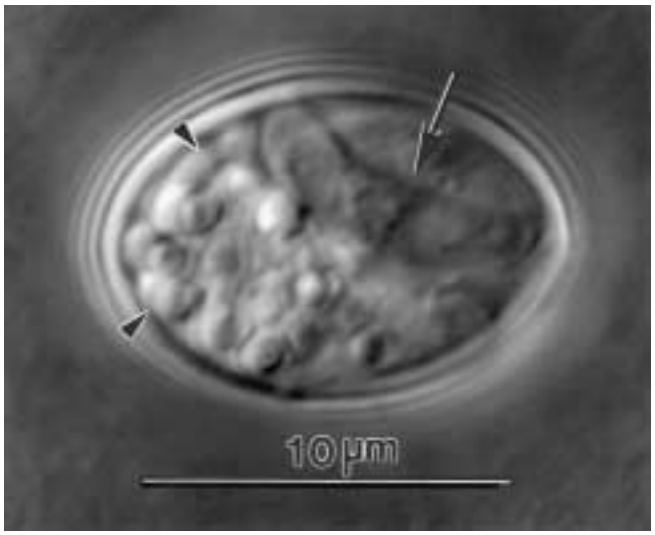

FIGURE 1. *Sarcocystis speeri* sporocyst from the feces of opossum no. 25 (*Didelphis virginiana*) fed sarcocysts derived from the Argentinian opossum (*Didelphis albiventris*). Note dispersed residual bodies (arrowheads) and an elongated sporozoite (arrow). Unstained.

TABLE I. Sarcocystosis in KO mice fed sporocysts from opossum no. 25 (experiment 1).*

Mouse no.	Dose	Day killed/died	Schizonts	Sarcocysts
4551	42,000	DK29	Brain	Sk, T
4552	42,000	DK33	Brain	Sk
4556	42,000	DK36	Brain	Sk, T
4557	42,000	D37	Brain	Sk, T
4558	42,000	DK29	Brain	Sk, T
4851	4,200	DK39	None	Sk, T
4852	4,200	D48	NE	
5128	420	K85	None	Sk, T
5129	420	D39	NE	

* K = killed; D = died; DK = killed when ill; NE = not examined; Sk = skeletal muscle; T = tongue.

the mice were killed 64 and 71 DAF (Dubey, Venturini et al., 2000). Part of the carcass from the KO mouse (no. 4217) killed 71 DAF was shipped cold to Cornell University for feeding to a captive opossum (no. 25). The opossum had no sporocysts in feces 32 days, and 1 day, before and days 0–10 after feeding the infected mouse carcass as determined by sugar flotation of feces. Sporocysts were seen in fecal floats on days 11–13; the opossum was killed 13 days after feeding (DAF), and its small intestine was shipped cold to Beltsville for parasitologic ex-

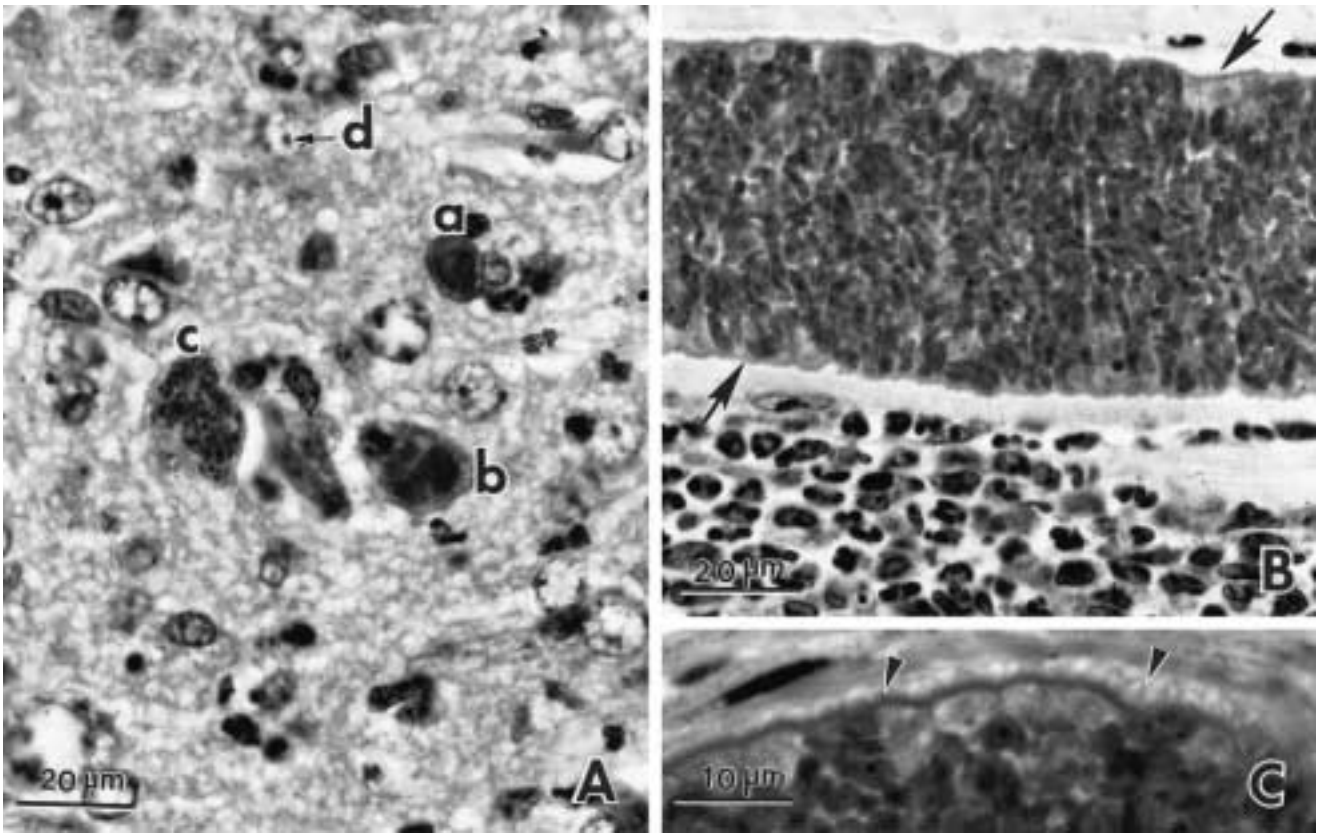

FIGURE 2. *Sarcocystis speeri* stages in sections of brain (A) and skeletal muscle (B, C) of KO mice fed sporocysts from opossum no. 25. HE stain. **A.** Cerebrum with an inflammatory focus. Note developing schizonts (a–c) and free merozoites (d). Mouse no. 4552, 33 DAF. **B.** Longitudinal section of a mature sarcocyst with numerous bradyzoites. The cyst wall has minute projections (arrows). Mixed cellular infiltrate surrounds the cyst at the bottom of the figure. KO mouse 5128, 85 DAF. **C.** Higher magnification of the cyst wall showing minute villar projections.

amination. At Beltsville, sporocysts were collected from the scrapings of the small intestine by homogenization in water. Sporocysts were suspended in antibiotic saline (Dubey et al., 1989; Dubey, Venturini et al., 2000).

Sporocysts (~420–42,000) from opossum no. 25 were fed to 9 KO mice (Table I) and 4 budgerigars. The mice were examined 29–85 DAF (Table I) and birds were killed 14 (2 birds)and 35 (2 birds) DAF.

Mice and birds that were killed or died were necropsied. Portions of all internal organs, eyes, and skin were fixed in 10% buffered neutral formalin and processed for histology. Paraffin-embedded sections were cut at 5 μm thickness and examined after staining with hematoxylin and eosin (HE). For immunohistochemical staining, paraffin sections were reacted with anti-*S. neurona* antibodies using techniques and reagents described previously (Lindsay and Dubey, 1989; Dubey et al., 1998; Dubey, Mattson et al., 1999). Antibodies to *S. neurona* had been obtained from rabbits immunized with the SN2 and SN6 isolates of *S. neurona* isolated from paralyzed horses (Dubey, Mattson et al., 1999). Tissues from a mouse experimentally infected with *S. neurona* and a budgerigar with *S. falcatula* (Dubey and Lindsay, 1998) were used as positive controls. Tissues were processed for transmission electron microscopy (Dubey et al., 1998).

In experiment 2, portions of carcasses from 11 KO mice fed sporocysts from a naturally infected *D. virginiana* (no. 47; Dubey et al., 1998) were fed to a captive opossum (no. 30) at Cornell University. The opossum shed sporocysts 12 days after ingesting sarcocysts. Sporocysts from opossum no. 30 were fed to 2 KO mice (nos. 4867, 4868) and 2 budgerigars (nos. 59, 60).

Sporocysts from opossums no. 25 (12–14 × 9–11 μm, n = 25) and no. 30 (12–14.5 × 9–10.5 μm; n = 25) were similar in size. Sporocysts contained residual bodies and had elongated sporozoites. A 9-μm-long sporozoite is visible in Figure 1.

Depending on dose, KO mice fed sporocysts from opossum no. 1 developed clinical sarcocystosis and 3 died 37, 39, and 48 DAF; 2 were too autolyzed for histologic evaluation and were discarded (Table I). Schizonts, sarcocysts, or both were found in tissues of mice killed 29–85 DAF (Figs. 2, 3); they were identical to those of *S. speeri* (Dubey et al., 1998; Dubey and Lindsay, 1999). Schizonts (Fig. 2A) in tissues of experimentally infected KO mice fed sporocysts from opossum no. 25 were similar to schizonts from mice fed sporocysts from *D. albiventris* (Dubey, Venturini et al., 2000) and *D. virginiana* (Dubey et al., 1998).

Ultrastructurally, sarcocysts from the KO mouse no. 5128 killed 85 DAF were identical to those from the KO mouse no. 4218 examined 64 DAF with sporocysts from the Argentinian opossum (*D. albiventris*) (Dubey, Venturini et al., 2000) and the KO mice fed sporocysts from the naturally infected opossum (*D. virginianus*) from the U.S. (Dubey et al., 1998). The primary sarcocyst wall had steeple-shaped villar projections (Fig. 3).

Schizonts and sarcocysts (Fig. 2B, C) in tissues of mice fed sporocysts from opossums no. 25 and no. 30 did not react with anti-*S. neurona* and anti-*S. falcatula* antibodies. Ultrastructurally, schizonts in the liver of KO mouse fed sporocysts from the Argentinian opossum were similar to the schizonts and merozoites of *S. speeri* derived from the North American opossum (Dubey et al., 1998)

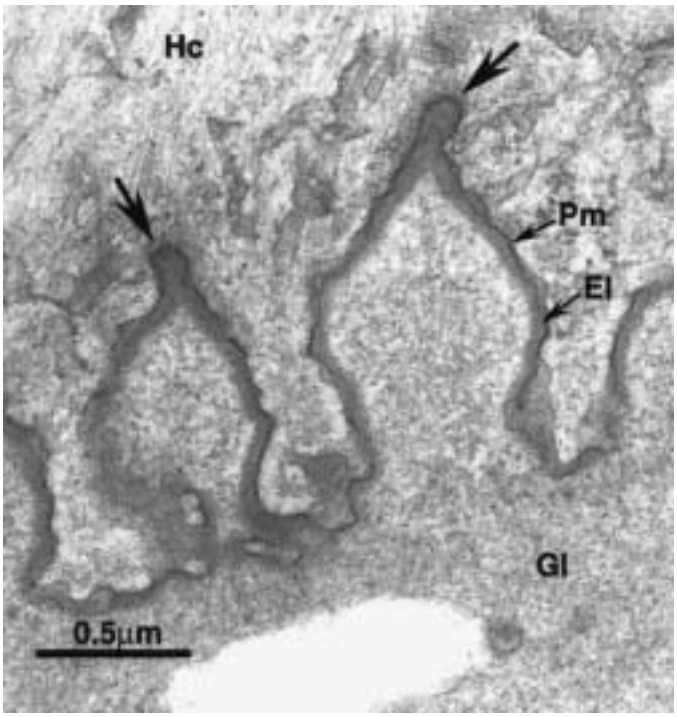

FIGURE 3. Transmission electron micrograph of the wall of a sarcocyst of *Sarcocystis speeri* in the KO mouse no. 5128 in Figure 1B. The primary sarcocyst wall consists of a parasitophorous vacuolar membrane (Pm) and an underlying electron-dense layer (El). Note the steeple-shaped villar projections that are surmounted by a spire (arrows); Gl, granular layer of wall; Hc, host cell cytoplasm.

Sarcocystis stages were not found in tissues of the 4 budgerigars killed 14 and 35 DAF, suggesting that *S. speeri* is not infective to budgerigars and distinct from *S. falcatula.*

In experiment 2, 1 of the 2 KO mice (no. 4868) died 51 DAF and mouse no. 4867 was killed 70 DAF; sarcocysts of *S. speeri* were found in muscles of both. The 2 budgerigars fed sporocysts from opossum no. 30 remained clinically normal and *Sarcocystis* stages were not demonstrable in their tissues. These studies support earlier results that *S. speeri* is not infective to budgerigars. Sporocysts from the intestines of experimentally infected opossums no. 30 and no. 25 were identical to sporocysts from the intestine of naturally infected *D. virginiana* no. 26 (Dubey et al., 1998). *Sarcocystis* stages in tissues of mice did not stain with anti-*S. neurona* antibodies. Results of the present study indicate that *S. speeri* occurs naturally in the South American opossum and is infective to mice but not to budgerigars; thus, *S. speeri* is distinct from *S. falcatula.*

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Prevalence of *Toxoplasma gondii* Antibodies in Sera of Turkeys, Chickens, and Ducks from Egypt

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ABSTRACT: Sera from 173 turkeys, 108 chickens, and 48 ducks from Giza, Egypt, were tested for the presence of anti-*Toxoplasma gondii* antibodies by means of the modified agglutination test using mercaptoethanol and formalin-fixed tachyzoites. The prevalence of anti-*T. gondii* antibodies (>1:25) among turkeys, chickens, and ducks was 59.5%, 47.2%, and 50%, respectively.

Birds and rodents are important intermediate hosts of *Toxoplasma gondii* because they serve as a source of *T. gondii* infection for cats (Dubey and Beattie, 1988). Cats excrete the environmentally resistant *T. gondii* oocysts in their feces after ingesting tissue cysts from infected animals. Viable *T. gondii* was found in 54% of 50 feral chickens caught around human dwellings in Costa Rica (Ruiz and Frenkel, 1980). In that study, *T. gondii* infection was detected by bioassay of chicken tissues in mice, because the Sabin–Feldman dye test does not detect antibodies in chicken sera (Frenkel, 1981). Recently, Dubey, Camargo, Ruff, Wilkins et al. (1993) and Dubey, Camargo, Ruff, Shen et al. (1993) demonstrated that the modified agglutination test (MAT) was highly sensitive and specific for detecting antibodies to *T. gondii* infections in chickens and turkeys.

Little is known of toxoplasmosis in turkeys and ducks. There are reports of fatal toxoplasmosis in 2 turkeys from the U.S. (Howerth and Rodenroth, 1985; Quist et al., 1995). Lindsay et al. (1994) found MAT antibodies to *T. gondii* in 12 of 17 sera from turkeys in the U.S. and they also isolated *T. gondii* from the hearts of 8 of 16 turkeys. Boehringer et al. (1962) reported fatal toxoplasmosis in a duck from Argentina. Literák and Hejlíček (1993) found antibodies to *T. gondii* in 5 (1.7%) of 297 ducks and isolated *T. gondii* from 1 of 60 ducks from the Czech Republic.

Because the prevalence of *T. gondii* in chickens, turkeys, and

TABLE I. Seroprevalence of *Toxoplasma gondii* in turkeys, chickens, and ducks in Egypt.

Animal species	No. of sera	No. with anti-*T. gondii* antibodies			Total seropositive (≥1:25)	Percent seropositive (≥1:25)
		1:25	1:50	1:500		
Turkeys	173	28	26	49	103	59.5
Chickens	108	25	4	22	51	47.2
Ducks	48	14	5	5	24	50.0